Cell Host & Microbe
Meeting Review

The Virulence of Human Pathogenic Fungi: Notes from the South of France

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The Second FEBS Advanced Lecture Course on Human Fungal Pathogens: Molecular Mechanisms of Host-Pathogen Interactions and Virulence, organized by Christophe d'Enfert (Institut Pasteur, France), Anita Sil (UCSF, USA), and Steffen Rupp (Fraunhofer, IGB, Germany), occurred May 2007 in La Colle sur Loup, France. Here we review the advances presented and the current state of knowledge in key areas of fungal pathogenesis.

The Second FEBS Advanced Lecture Course on Human Fungal Pathogens: Molecular Mechanisms of Host-Pathogen Interactions and Virulence brought together investigators and students from across the globe to a beautiful sunny setting in Provence, France. This course, which was attended by 40 investigators, both European and non-European, and approximately 130 students provided a state-of-the-art introduction to key concepts of human fungal pathogenesis.

Fungi are important infectious agents of both immunocompetent and immunocompromised individuals. Therefore, as the population of immunosuppressed individuals has increased secondary to HIV infection, cancer/chemotherapy, organ transplantation, or autoimmune disorder, the incidence of fungal disease has surged. For instance, the yeasts *Candida* are the fourth most common pathogens isolated from nosocomial bloodstream infections (Edmond et al., 1999). The human pathogenic fungi are broadly classified into two groups: the commensals, such as *Candida spp.*, dermatophytes, and *Malassezia spp.*, which are normal constituents of the human microflora and generally cause disease in the setting of altered host defenses, and the environmental pathogens, such as *Cryptococcus neoformans*, and the thermally dimorphic fungi (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, *Penicillium marneffei*, and *Sporothrix schenckii*) (Figure 1), which reside in specific environmental niches, and humans are exposed by inhaling spores or small yeast cells. The spectrum of fungal disease varies widely from cutaneous skin or nail infections to life-threatening disseminated disease. Despite an increasing incidence of fungal infections and significant morbidity and mortality, treatment strategies are often ineffective due to delays in treatment caused by suboptimal diagnostics, undesirable side effects and drug-drug interactions, and increasing incidence of antifungal resistance among pathogens. Although this meeting covered an array of human fungal pathogens, several themes and key topics emerged, including how virulence has evolved in pathogenic fungi, the role of dimorphism in virulence, the dynamic interplay between host and pathogen, new antifungal strategies, and the power of genomics and studies of sexual reproduction in human pathogenic fungi. This report offers an opportunity to review the state of the field and discuss these areas of fungal pathogenesis.

Evolution of Virulence in Microbial Pathogens

Of threats to human health, none are as pervasive and myriad as infectious diseases. How microbes evolve to interact with human hosts, in commensal and disease states, is a key question in all fields of microbial pathogenesis. Arturo Casadevall (Albert Einstein, USA) advanced two hypotheses for the evolution of fungal pathogens in an opening plenary lecture. The first hypothesis posits that some fungal pathogens have specifically evolved to interact with the human host. In support of this theory are clear examples in which virulence attributes may have evolved in response to human-human, animal-human, or animal-environment-animal/human cycles. For this group of organisms that includes *Candida spp.*, virulence reflects a disruption of the host-microbe relationship. The second hypothesis suggests that human encounters with fungal pathogens are entirely accidental, and that these are not coevolved human pathogens. Rather, the capacity for mammalian virulence for these fungi evolved in heterologous environmental hosts, including amoeba, slime molds, insects, and plants, which selected for broad host range virulence determinants that inadvertently enabled survival in human hosts (Steenbergen and Casadevall, 2006).

Evidence for the specific evolution of fungal pathogens to human hosts derives from studies of *Pneumocystic jirovecii* (Melanie Cushion, University of Cincinnati, USA; Moira Cockell, CHUV, Switzerland) and the Microsporidia. Infection with *Pneumocystis* occurs via the respiratory tract, presumably via human-human transmission like *Mycobacterium tuberculosis*. *Pneumocystis* occurs as several closely related host-specific species, and these are only known to proliferate in lungs of infected mammals. The absence of any known environmental

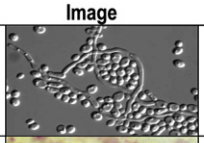
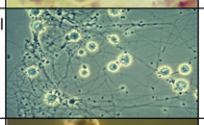
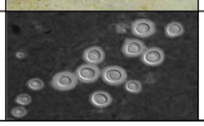
Organism	Source	Image	Disease Spectrum
Candida spp.[1]	Commensal of the skin, GI tract, and vagina.		•Hematogenously disseminated infections •Mucocutaneous infections: Oropharyngeal infections (thrush) Skin/nail infections Vaginitis
Pneumocystis spp.[2]	Species are host specific, with no known environmental reservoir.		•Pneumonia
Histoplasma capsulatum[3]	Found in the environment in soil contaminated with bird or bat guano. Humans infected by inhaling conidia (spores).		•Self-limited flu-like syndrome •Acute pneumonia •Chronic pulmonary infection •Disseminated disease
Blastomyces dermatitidis[4]	Found in the environment in soil / decaying wood. Humans infected by inhaling conidia (spores) or direct inoculation of the skin.		•Acute/chronic pulmonary infections •Skin lesions/ subcutaneous nodules •Disseminated disease
Malassezia spp.[5]	Commensal of the skin.		•Primarily cutaneous infections Pityriasis versicolor Seborrheic dermatitis •Allergic atopic eczema
Cryptococcus spp.[6]	Found in the environment in association with soil, pigeon guano, and trees. Infection occurs through inhalation of spores or small yeast.		•Pneumonia •Meningitis

Figure 1. Representative Human Pathogenic Fungi Presented at the Meeting

For each fungus, the source and disease spectrum are described along with an accompanying image.

(1) *C. albicans* yeast and hyphal cells. Adapted by permission from Macmillan Publishers Ltd: *Nature Reviews Genetics* (Berman and Sudbery, 2002).

(2) Light microscopy of Diff-Quick-stained *Pneumocystis wakefieldiae* depicting five cysts. The top left cyst is a mature ascus containing all eight ascospores. The two cysts on the right represent immature forms containing naked nuclei. The remaining cysts are mature forms in various stages of excystment. Picture courtesy of Melanie Cushion (University of Cincinnati, USA).

(3) Phase contrast microscopy of *Histoplasma capsulatum* mycelial (environmental) form depicting hyphae and conidia. Courtesy of http://www.doctorfungus.org/ (©2007).

(4) Yeast form cells of *Blastomyces dermatitidis*. Reproduced from *The Journal of Experimental Medicine*, 1999, Volume 189, issue number 8; issue cover image (©1999; The Rockefeller University Press).

(5) Periodic Acid Schiff (PAS) staining of *Malassezia* in a skin biopsy specimen from a patient with seborrhoeic dermatitis. Courtesy of Annika Scheynius (Karolinska Institutet, Stockholm, Sweden).

(6) India ink preparation of *C. neoformans* highlighting the polysaccharide capsule surrounding the yeast cells. Courtesy of Alexander Idnurm (Duke University, USA).

reservoir suggests that human-human transmission provides selective pressure to maintain virulence attributes for mammalian infection. Similarly, Microsporidia require the safe harbor of an infected mammal. In contrast to *Pneumocystis*, Microsporidia have dramatically reduced genomes (~3.5 MB) and likely derive key factors for survival from the host (Keeling and Slamovits, 2004). Transmission occurs from human to human, providing selective pressure for the maintenance of virulence.

The dermatophytes (such as *Trichophyton spp.*) and skin-associated commensals (such as the yeast *Malassezia*) are perhaps the most successful human fungal pathogens. As the causes of athlete's foot and skin, scalp, and nail infections, dermatophytes are ubiquitous and afflict the majority of the population. These fungi spread via both direct human-human contact and infected fomites and are specialized to utilize nutrients available from the human body, including keratin and lipids in sebaceous secretions. They evade immune detection by residing in poorly vascularized tissues of lower temperature or in which immunity is less effective. These fungal pathogens therefore likely evolved to specifically colonize mammalian hosts.

Human-to-human transmission has been documented for *Candida spp*., particularly *Candida parapsilosis* (Geraldine Butler, University College Dublin, Ireland), a common commensal of the skin that can be transferred directly from person to person, best characterized in the setting of health care workers to patients. Transmission also occurs via oral-oral or fecal-oral transmission and can lead to disease in a variety of settings, including systemic disease in neutropenic hosts, oropharyngeal infection (thrush) in the setting of AIDS, and vaginitis in otherwise healthy women. Thus, in four quite divergent genera, *Candida*, *Pneumocystis*, Microsporidia, and the dermatophytes/skin commensals, human fungal pathogens have evolved in concert with mammalian hosts, suggesting that for these pathogens virulence is not an accidental encounter, but an evolved trait.

In contrast to the human commensal fungi, there is a panoply of environmental pathogenic fungi. In these cases, virulence may result from accidental host encounters, or from virulence evolution in heterologous hosts.

These organisms include filamentous fungal molds (*Aspergillus fumigatus*), pathogenic yeasts (*Cryptococcus neoformans*, *Cryptococcus gattii*), and the dimorphic fungal pathogens (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, *Penicillium marneffei*, *Sporothrix schenckii*). These environmental pathogenic microbes share unifying features, including occurrence in specific environmental niches (soil, trees, bats, guano), and the fact that humans are exposed by inhaling spores or small desiccated yeast cells, leading to an initial pulmonary infection.

Initial encounters between these fungi and humans may have been accidental, but their sophisticated interactions with the host, from sensing carbon dioxide to facultative intracellular survival in phagocytic immune cells, suggests that their successful virulence strategies are under selective pressure. Whether this results from convergent evolution to survival in heterologous hosts and mammals (the dual-use virulence factor hypothesis [Casadevall et al., 2003]) or selection via animal-animal or animal-environment-animal transmission remains to be explored. These examples highlight how little is known about the natural ecology of these organisms and how virulence evolved and is maintained.

Genomics

Advancements in deciphering the molecular processes that lead to virulence during fungal infections have been hampered by difficulties in manipulating the diverse fungal species that colonize and infect human hosts. Development of DNA transformation systems for fungal organisms single-handedly catapulted the field of fungal pathogenesis into the molecular era. More than 20 pathogenic fungal species can be manipulated by direct DNA transformation or *Agrobacterium tumefaciens* transconjugation. Availability of the complete genome sequence for major agents of human mycoses (*C. albicans*, *C. neoformans*, *Aspergillus fumigatus*, *Coccidioides immitis*, *H. capsulatum*) propelled the field into the genomics and postgenomics era, providing new tools for genome-wide and comparative genomic analysis. Genomics has also had great impact among fungal pathogens that are recalcitrant to DNA transformation and thus have been excluded from the advances of the molecular era.

The power of genomics for intractable species was exemplified by studies from Melanie Cushion's group (University of Cincinnati, USA) on *Pneumocystis carinii* (Cushion et al., 2007). *Pneumocystis spp.* are pathogens that reside in alveoli of mammalian hosts and are the most common etiological agents of acute fungal pneumonia in immunocompromised individuals. Causative factors associated with *Pneumocystis* mycoses are challenging to decipher because *Pneumocystis* species cannot be cultured. Analysis of an EST library constructed from *P. carinii* organisms harvested from rats with fulminant pneumonia revealed a high abundance of Major Surface Glycoprotein (MSG) transcripts. These *Pneumocystis*-specific genes comprise a glycoprotein family thought to facilitate escape from immune detection. They also have adhesion properties enabling cell-cell interactions and host cellular adhesion. Additional analysis revealed an abundance of transcripts involved in metabolic functions, suggesting that *P. carinii* may be capable of survival without scavenging host resources. Interestingly, several genes related to the sexual cycle were identified, suggesting that sex may occur in the mammalian lung during infection. The ability of *P. carinii* to sustain metabolism and undergo sexual reproduction inside the host resembles plant biotrophic fungi. These fungi complete their entire life cycle in plants and are incapable of ex vivo growth. This resemblance led Cushion to postulate that, like biotrophs that are "compatible" with their hosts, a similar interrelationship can be applied to *Pneumocystis*. Without the need to damage the host during nutrient acquisition, *Pneumocystis* has evolved a sustainable or "compatible" relationship with the host. However, under conditions that lead to immunosuppression, this relationship is altered, resulting in disease.

In addition to serving as a platform to study intractable fungi, genomics provides insights into more experimentally amenable fungi. The virtues of genomics were illustrated by Malcolm Whiteway (McGill University, Canada) in studies of the regulatory circuitry controlling galactose utilization in *C. albicans* (Martchenko et al., 2007). In the related fungus *Saccharomyces cerevisiae*, the transcriptional regulation of galactose-metabolizing genes is controlled by an upstream activating sequence, UAS_{Gal4}, that recruits the transcriptional activator Gal4. By comparative genomics, Whiteway's group found that, although the *C. albicans* galactose utilization gene homologs are syntenic, the upstream regulatory sequences have diverged, and the *C. albicans* UAS_{Gal4} sequence directs the transcription of a different gene set. Furthermore, transcriptional induction of galactose-metabolizing genes requires the transcription factor Cph1 and is independent of CaGal4. Thus, although *S. cerevisiae* and *C. albicans* have maintained similar machinery for galactose metabolism, the regulatory circuitry has diverged, resulting in a transcriptional "rewiring," possibly as *C. albicans* adapted to the human host. Comparative genomics, not only of promoter sequences, but also of whole genomes, promises to be a robust tool for global comparisons of gene families and signaling pathways to identify both conserved and novel biological processes in closely related species.

Sex and Evolution

How pathogenic fungi undergo sexual reproduction has undergone a renaissance as genome sciences allowed the identification of mating-type loci (*MAT* or *MTL*) and machinery for mating and meiosis. *C. albicans* was thought to be strictly asexual for more than a century, and yet with the identification of the *MTL* and conditions that support mating, it is clear that this organism is more sexual than anticipated. Geraldine Butler (University College Dublin, Ireland) and J.L.R. (Duke University, USA) examined genomes of other *Candida* species with complete sexual cycles including meiosis, *C. lusitaniae* and *C. guilliermondii*, emerging causes of infections in humans. The

MAT locus was defined, revealing conserved gene content throughout the *Candida* species, including three novel genes encoding a predicted poly A polymerase, an oxysterol-binding protein, and phosphatidylinositol-4 kinase. All of the machinery for sexual reproduction is maintained, but key elements predicted to be essential for meiosis are missing from all *Candida spp.*, including the two species with complete meiotic sexual cycles, and also in *C. albicans*, which is thought not to undergo meiosis. Thus, either these components are dispensable or these species undergo a parasexual reduction of their genomes, with potential low levels of recombination.

Sexual reproduction is thought to be a crucial part of the infectious life cycle for some fungal pathogens, particularly for the environmental fungi such as *C. neoformans.* This organism is found in association with pigeon guano, soil, and trees, and humans are exposed by inhaling small particles either as desiccated yeast cells or spores produced via mating in the environment. A novel gradient centrifugation approach to purify spores was developed (Christina Hull/Steven Giles, University of Wisconsin-Madison, USA), allowing detailed genetic, morphological, and virulence studies of spores. These studies revealed that spores can be more infectious than yeasts in some virulence studies. Spores are readily phagocytosed by macrophage cell lines, even without added opsonins, in contrast to yeast cells. Similarly, studies were presented that conidia (asexual spores) from the dimorphic pathogen *H. capsulatum* (Anita Sil/Charlotte Berkes, UCSF, USA) are phagocytosed by macrophages, and an interferon response more typical of viral infection is induced in host immune cells. Novel therapeutic approaches involving interferons already in clinical use for viral infections could be a possibility.

For the environmental fungi, sex may play an important part in the life cycle to generate the infectious particles that are inhaled by humans. However, for some commensal fungal species, such as *Candida*, there appears to be a negative correlation between the ability to complete a full meiotic sexual cycle (including spore formation) and success as a pathogen. For instance, *C. albicans*, *C. parapsilosis*, and *C. glabrata* are the most prominent *Candida spp.* causing infection, but all three lack complete sexual cycles. In contrast, *C. lusitaniae* and *C. guilliermondii* possess complete sexual cycles but are less frequent causes of candidiasis. Interestingly, the dermatophytic fungi also show a similar trend, suggesting that meiosis may actually be disadvantageous to certain pathogens. Whether the absence of a complete meiotic sexual cycle with spore formation is a trait that occurred as a prelude to human-human transmission and virulence is an open question.

Environmental Sensing and Morphogenesis

A common trait shared by environmental pathogenic fungi is the induction of a morphogenic transition upon infection of the mammalian host. Whether this transition is an evolved trait or not remains to be determined. However, this poses another fundamental question central to fungal pathogenesis: does morphogenesis promote virulence?

A variety of human fungal pathogens can grow in multiple morphological forms (commonly as yeast or hyphae). The human primary fungal pathogens (*Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Paraccocidioides brasiliensis*, and *Coccidioides immitis*) exist as mycelia (the hyphal form) in the environment; however, inhalation of conidia (asexual spores) by the host results in a temperature-induced mycelium-to-yeast transition. Hence, these species are referred to as thermally dimorphic fungi. This morphological transition is a critical prelude to infection, as Bruce Klein (University of Wisconsin, USA) demonstrated through work on a key regulator of the dimorphic transition in *Blastomyces dermatitidis*. Using an *Agrobacterium tumefaciens* T-DNA insertional mutagenesis approach, his laboratory identified a histidine kinase gene (Drk1), homologous to *Saccharomyces cerevisiae* Sln1, which when ablated locked *B. dermatitidis* in the mold phase. Virulence studies demonstrate that strains lacking Drk1 are attenuated, and *DRK1* silencing in *Histoplasma capsulatum* also reduced virulence (Nemecek et al., 2006), providing evidence linking morphogenesis to virulence.

The commensal fungus *Candida albicans* is also dimorphic. In contrast to the thermally dimorphic fungi, both yeast and hyphae of *C. albicans* are found in the infected human host. While the hyphal phase is associated with tissue adhesion and invasion, the yeast phase is thought to be important for dissemination during systemic infection. As discussed by Alistair Brown (University of Aberdeen, UK), key genes that regulate dimorphic transitions in *C. albicans* have been identified, and genetic ablation results in strains locked in the yeast (*cph1/cph1 efg1/efg1* strains) or (pseudo)hyphal phase (*nrg1/nrg1* and *tup1/tup1* strains). Virulence studies in a murine systemic infection model revealed that both types are avirulent (Lo et al., 1997; Murad et al., 2001; Saville et al., 2003), suggesting both budding and filamentous morphotypes are important for pathogenesis. However, caution is warranted with this interpretation, since *EFG1*, *CPH1*, *NRG1*, and *TUP1* regulate other virulence factors not linked to morphogenesis. The hyphae-specific cyclin gene *HGC1* was recently shown to be required for hyphal development in *C. albicans* (Zheng et al., 2004). Under all conditions tested, *hgc1/hgc1* mutants failed to form hyphae and exhibited attenuated virulence. Further analysis showed that Hgc1 interacts with the Cdc28 kinase, suggesting that Hgc1 promotes hyphal development by regulating apical elongation. As with *B. dermatitidis*, these findings provide further evidence that morphological transitions are required for pathogenicity of dimorphic fungi.

The interplay between host signals and dimorphic transitions has been elegantly demonstrated in *C. albicans*. During adaptation to different host niches, *C. albicans* has evolved sophisticated mechanisms for sensing and responding to various environmental factors (Biswas et al., 2007). One mechanism is its ability to respond to low oxygen levels (hypoxia) in the anaerobic environment of the gastrointestinal tract. As presented by Siobhan

Mulhern (University College Dublin, Ireland), *C. albicans* adaptation to hypoxic conditions relies on the yeast filamentous transition. This transition is dependent on the transcription factor Ace2, which regulates genes involved in cellular respiration and filamentous growth under hypoxic conditions (Mulhern et al., 2006). Induction of *C. albicans* filamentation during hypoxic conditions provides another example of the importance of morphogenesis during host colonization. In conclusion, mounting evidence argues strongly for a role of dimorphism during fungal pathogenesis (Rooney and Klein, 2002), providing a promising therapeutic avenue for treatment of human mycoses.

Host-Pathogen Interactions

Understanding the molecules mediating interactions at the host-fungal interface is crucial to developing new approaches for treatment and management of fungal infections. From the pathogen's perspective, this includes identification of key virulence factors that allow the pathogen to cause disease (such as morphogenesis) or to escape immune detection by subverting innate immunity. Numerous species-specific virulence factors have been described, such as the ability of *Cryptococcus* to elaborate a polysaccharide capsule and produce melanin (Guilhem Janbon, Institut Pasteur, France; Arturo Casadevall, Albert Einstein, USA) (Casadevall and Perfect, 1998). Other virulence factors, such as the ability to grow at human body temperature and adherence to host tissues, are universal among fungal pathogens, although the specific molecules and pathways involved often differ. Conversely, from the host perspective, this involves elucidating the mechanisms by which the host recognizes and responds to fungal pathogens, resulting in containment of infection, or in some cases, an overzealous immune response, leading to allergy and inflammation.

The damage response network hypothesis was presented by Arturo Casadevall (Albert Einstein, USA) as a model to conceptualize host-fungal interactions (Casadevall and Pirofski, 2003). According to this hypothesis, there are two primary host immunological states under which disease occurs. The first disease state results from a weak immune response. This is the case for many fungal pathogens, such as *Cryptococcus* and *Aspergillus*, which cause disease primarily in patients with immunodeficiencies secondary to transplantation, cancer, or HIV infection. However, a weak immune response can also result from the fungal pathogen's ability to camouflage or shield itself from immune recognition (Neil Gow, University of Aberdeen, UK). The second disease state occurs in the presence of an overzealous immune response to a fungal pathogen, such as occurs in patients with recurrent vulvovaginal candidiasis (Fidel, 2007), immune reconstitution syndrome with *Cryptococcus* (Singh et al., 2005), allergic reaction to *Aspergillus*, or sensitization to *Malassezia spp.* in the setting of atopic eczema (Schmid-Grendelmeier et al., 2006).

Numerous tools and models have been harnessed to elucidate the host-fungus interaction and to learn how the host recognizes and clears fungal pathogens (Neil Gow, University of Aberdeen, UK). The utilization of model host systems (*Drosophila melanogaster*, *C. elegans*, the wax moth *Galleria mellonella*, mammalian models [mice, rabbits, rats], human cell lines, and human reconstituted/model tissues) to study fungal-host interactions (Casadevall, 2005; Schaller et al., 2006) has resulted in numerous discoveries, including the identification of Toll-like receptors involved in innate immune recognition of fungal pathogens (Lemaitre et al., 1996).

Insight into how the immune system recognizes fungal pathogens has also revealed mechanisms by which these pathogens evade host immunity. Work presented by Gordon Brown (University of Cape Town, South Africa) demonstrated that the C-type lectin Dectin-1 is expressed by multiple immune effector cells and recognizes β-glucans (a fungal cell wall component) to mediate binding, uptake, and killing of fungal pathogens (Brown, 2006). Interestingly, coincident with the morphological transition from mycelial to yeast growth upon infection of the human host, thermally dimorphic fungi, such as *H. capsulatum*, change their cell wall composition from primarily β-glucan to predominately α-(1-3)-glucan. Elaboration of cell wall α-(1,3)-glucan is positively correlated with pathogenicity, but the reason was previously unknown. With new insights into how the immune system recognizes fungal pathogens, William Goldman's laboratory found that incorporation of α-(1,3)-glucan into the *H. capsulatum* cell wall conceals β-glucans from detection by Dectin-1 (Rappleye et al., 2007). Thus, understanding how the host senses fungi provides insight into how pathogens subvert normal immune responses resulting in a weak immune response.

Fungal disease can also result from an overzealous immune response, as illustrated by Annika Scheynius's analysis (Karolinska Institutet, Sweden) of the role that *Malassezia* species play in the immune disorder atopic eczema (Schmid-Grendelmeier et al., 2006). Atopic eczema is a chronic inflammatory skin disorder that develops when defective skin barriers permit entry of environmental factors into cutaneous tissues, where they act as allergens provoking an inappropriate inflammatory response. Yeasts such as *Malassezia* are common skin commensals in a majority of the population. Patients with atopic eczema mount an exaggerated allergic immune response to *Malassezia* that is not seen in patients with other allergic diseases or normal hosts (Casagrande et al., 2006). Thus, this normally commensal organism contributes to pathogenesis by provoking allergic inflammation. Treatment with the antifungal drug ketoconazole improves eczema, suggesting that commensal fungi have an intimate role in disease development. This clearly links a commensal microbe to an overzealous, rather than deficient, immune response resulting in disease. An understanding not only of the pathogen, but also of how the host responds to fungal pathogens, is crucial for the development of new treatment strategies.

Antifungal and Vaccine Strategies

Of the microorganisms causing disease in humans, both fungi and parasites are eukaryotic cells similar to our

own cells, and thus finding suitable targets for antifungal therapy is challenging. The current antifungal armamentarium is limited, and treatment is complicated due to side effects and drug-drug interactions. Current research on antifungals focuses on three primary areas as described by Dominique Sanglard (CHUV, Switzerland) in an introductory talk: strategies to increase the efficacy of existing antifungals, developing novel therapies, and understanding drug resistance.

Mortality from fungal infections increases with delayed treatment; thus, empirical treatment is often initiated before culture-based identification. Improving current antifungal therapeutics aims at developing diagnostic tools such that causative agents can be identified earlier and targeted treatment begun. Other research focuses on novel drug combinations that could enhance efficacy of available antifungals. One example is the azoles, normally fungistatic drugs, which become fungicidal when combined with calcineurin inhibitors (FK506 and Cyclosporine A) (Steinbach et al., 2007). Although immunosuppressive side effects of calcineurin inhibitors may limit systemic application, some promise has been shown in treating fungal eye and skin infections in model systems, settings in which systemic side effects may be mitigated in patients (Onyewu et al., 2006; Steinbach et al., 2007).

Other research focuses on developing novel antifungal agents, including panfungal vaccines for patients at high risk of fungal infections. The development of an antifungal vaccine is a new concept, since previous dogma suggested that to resolve fungal infections the host needed to mount a Th-1 T cell-mediated immune response (Romani, 2004). The majority of individuals develop antibodies to organisms such as *C. albicans* and *C. neoformans* early in life; however, these polyclonal antibodies do not always provide subsequent protection. One explanation is that only some antibodies are beneficial, whereas others could exacerbate disease (Cutler et al., 2007). Current research focuses on developing monoclonal antibodies to fungal epitopes that may enhance immune response by mechanisms including neutralization of fungal virulence factors or direct killing, opsonization, and stimulation of cell-mediated immunity. Antonio Cassone (Instituto Superiore di Sanita, Italy) outlined the current state of antifungal vaccine development. Various fungal proteins (*C. albicans* Hsp90 and Als1, *H. capsulatum* Hsp60 and histone-H2B-like protein, and *B. dermatitidis* Bad1) and carbohydrate antigens (*C. neoformans* capsule component, GXM, and *C. albicans* short-chain β-1,2-linked oligomannosides) have been utilized with variable results. However, these antigens are relatively species specific, and thus multiple vaccines would be required. β-1,3-glucan, a primary component of the fungal cell wall, has been proposed as a panfungal vaccine candidate; initial studies suggest that protective antibody responses can be generated (Rachini et al., 2007). Although fungal vaccines are advancing, there were questions raised during discussions at the conference, including the efficacy of these vaccines in immunodeficient hosts and potential consequences of developing a vaccine to a commensal, such as *C. albicans*. This highlights our limited knowledge concerning roles of *Candida* in the host in the nondisease state.

The development of new antifungal strategies is needed as resistance to current therapies emerges. Many mechanisms of antifungal resistance have been characterized (as discussed by Ted White, SBRI, USA), including upregulation of efflux pumps and target genes, mutation of targets that reduce drug binding, or the elaboration of fungal biofilms that are less susceptible to drugs. However, novel mechanisms of antifungal drug resistance continue to be discovered. For instance, Judith Berman (University of Minnesota, USA) presented a novel mechanism of antifungal resistance acquisition through the formation of an isochromosome in response to fluconazole (Selmecki et al., 2006). Using comparative genome hybridization, multiple fluconazole-resistant isolates were identified with an increased copy number of the left arm of chromosome 5, which results from formation of an isochromosome possessing two chromosome 5L arms flanking a single centromere. There was a concomitant increase in expression level of most genes on chromosome 5L, including two key players in fluconazole resistance: *ERG11* (encoding the target of fluconazole, lanosterol-14α-demethylase) and *TAC1* (encoding a transcription factor that upregulates expression of ABC transporters, Cdr1 and Cdr2, that efflux fluconazole). The utilization of a genomics approach to studying antifungal resistance highlights the dynamic nature of the *C. albicans* genome and how genome plasticity can confer antifungal resistance.

Conclusions and Outlook

The field of fungal pathogenesis has grown exponentially over the past decades, particularly with the aid of complete genome sequences that have allowed multiple genome comparisons and insight into genetically intractable fungi. With more fungal genomes currently being assembled (including *Pneumocystis*, dermatophyte, and *Malassezia* species), the impact of genomics will continue to grow. The expanding use of model host systems holds promise for furthering our understanding of how the innate immune system recognizes fungal pathogens, as well as the virulence factors that fungi use to subvert the immune response and cause disease in human hosts. However, there are many questions that remain to be answered, including understanding the nature of the infectious propagule for fungi such as *Cryptococcus* and the dimorphic fungal pathogens normally found in the environment. With relatively few new antifungal drugs coming to market (one new class within the past 25 years), and an increase in organisms resistant to available therapies, there is an urgent need to develop new therapeutics including drug combinations, selective immune reconstitution, and vaccines. From studies presented at the meeting, the field is advancing in elucidating virulence-associated mechanisms, but less is appreciated about commensal relationships on skin, the GI tract, and other mucosal surfaces. As the NIH-supported human microbiome program advances, a focus should be examination of the fungal

microbiome, and its relationship with bacterial microbiota and host in both commensal and disease states.

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